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September 7, 2004

TO: Dr. Gary Benzion
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FROM: Khue Nguyen, Ph.D.
Tel. (619) 543-3623
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RE: Patent Application # 09/938,013

Please find attached the following:

- Copy of Sep 7, 2004 Fax to Ms. J. Goldberg
- Copies of previous faxed communications to Ms. J. Goldberg: Dec. 9, 2003 Fax, May 24, 2004 Fax and May 28, 2004 Fax.

Total fax pages: 13

September 7, 2004

TO: Ms. Jeanine Goldberg FAX: 571-273-0743
U.S. Patent & Trademark Office

FROM: Khue Nguyen, Ph.D.
Tel. (619) 543-3623
FAX (619) 543-7868

Cc: Dr. Gary Benzion, USPTO
Dr. Mai Nguyen

RE: Patent Application # 09/938,013

This is to confirm our telephone conferencing appointment with you, Dr. Benzion, Mai Nguyen (tel. 760/438-5083) and Khue Nguyen (tel. 619/543-3623) on Sep. 9, 2004, at 12 noon Eastern time (9:00 am California time).

To facilitate our conferencing, please find attached a draft of some of the main points that I hope could be addressed.

Total fax pages: 3

Following are some of the points and issues I hope you would clarify and address for me.

A/ I do not understand why you still maintain the issue of new matter in the last Office communication (6/4/2004) while you had already accepted my clarification that there is no new matter as per my phone discussion with you on 1/20/2004 and following my 12/9/2003 FAX. On July 15, 2003 you said that you would not pursue the examination of the application unless the issue of new matter is resolved; the fact of the matter is that you have accepted that there is no new matter by the very fact that you have continued to examine my application.

B/ After my clarification for you regarding the difference between's Jong's method and my method (refer to 5/24/2004 FAX and 5/26/2004 phone conferencing, see 5/28/2004 FAX) you accepted that my work is novel. During the phone conferencing, you talked about the known techniques that can be applied to all kinds of disease; I pointed out that if that is the case, then we would have found the cure all the diseases of the world; so you then agreed to focus your consideration of the application as applied to the SMA disease. I do not understand why, in the 6/4/2004 Office communication , you however talked about the various techniques used for various purposes but not specifically applied for SMA diagnostic purpose.

C/ It is not clear as to why sometimes you stated that Jong's method is semi-quantitative (pages 11, 14, 16 of 6/4/2004 Office communication) and sometimes you stated that Jong's method is quantitative by stating that "the ratio is a quantitative method" (page 11 of 6/4/2004 Office communication), and by saying that "Jong teaches performing quantitative analysis by determining the ratio of exon 7..." (page 15 of 6/4/2004 Office communication) which is a misunderstanding on your part because the ratio has nothing to do with characterizing the type of method used in order to get the results of the experiments; the research method used can be qualitative, semi-quantitative or quantitative. In fact, the ratio is a means to correct the fluctuations of the PCR reaction rate to compare the results from one sample to another. In each sample, each result obtained from the PCR product to amplify exon 7 of the SMN gene is compared to the obtained result from the positive internal control, i.e., used as an internal referent, in order to estimate the number of transcripts containing exon 7 (or lacking exon 7). For example, in sample 1, the PCR reaction may give the amplification number 20 times and the internal referent may give the number 10 times; in sample 2, the PCR reaction may give the amplification number 10 times, and the internal referent may give the number 5 times. Researchers do not use the raw value of 20 in sample 1 nor the raw value of 10 in sample 2 to make comparisons because it gives inaccurate information (due to PCR reaction rate varying from one sample to another); researchers use the ratio 20/10 in sample 1 to compare with 10/5 in sample 2. Moreover, the more stable the PCR reaction rate of the internal referent is across samples, the more accurate the comparison would be possible.

In Jong et al.'s article, these researchers specified that they used the semi-quantitative method based on the intensity of the band of the PCR product, i.e., looking at the darker or lighter shade of the bands in order to estimate the results obtained; then, from the obtained estimated results, Jong et al. performed a ratio to make the comparisons.

In brief, it is unclear as to what your interpretation of the cited U.S.C. 103 (a) clause is and how you connect that to my specific case; you indicated that any ordinary artisan would have done this and that, while the fact of the matter is that no one before the date of this application has thought of nor successfully combined the various techniques to develop the quantitative method for molecular diagnosis of SMA at the mRNA level; no one before my application has used probes to detect SMA at the mRNA level. It is unclear as to how U.S.C. 103 (a) is interpreted and applied while you acknowledged that the development of a quantitative method would be an improvement of Jong's semi-quantitative method (page 16 of the 6/4/2004 Office communication).

TO: Ms. **JEANINE GOLDBERG**
USPTO Art Unit 1634

FAX# (703) 746-5149
December 9, 2003

FROM: Khue Nguyen, Ph.D. and
H- Mai Nguyen, Ph.D.

RE: Response draft on the issue of new matter, Application #09,938,013

This is an initial draft to facilitate our discussion over the phone when we call you. The following is to explain that there was no new matter brought up, which relates to your question of what portions of exons 7 and 8, i.e. what constitutes probes 1 and 2; probe 3 is also addressed.

In the field of molecular biology, when forward primer's and reverse primer's positions from which number to which number of nucleotides are specified, it refers to what portions of the gene is to be used.

Probe 1:
The specified positions of the oligonucleotide (g), forward primer position from 869-889 and the oligonucleotide (h), reverse primer position from 901-921 of the exon 7 of the SMN gene, refer to the portions of exon 7 of the SMN gene; therefore, based on such information, persons in the field of molecular biology would know exactly that the sequence fragment from 869-921 of exon 7 of the SMN gene is to be amplified in order to construct probe 1.

Probe 2:
The specified positions of the oligonucleotide (i), forward primer position from 922-941 and the oligonucleotide (d), reverse primer position from 957-976 of the exon 8 of the SMN gene, refer to the portions of exon 8 of the SMN gene; therefore, based on such information, persons in the field of molecular biology would know exactly that the sequence fragment from 922-976 of the exon 8 of the SMN gene is to be amplified in order to construct probe 2.

Probe 3:
The specified positions of the oligonucleotide (e), forward primer position from 672-690 and the oligonucleotide (f), reverse primer position from 705-723 of the HUMEF1AB gene, refer to the portions of HUMEF1AB gene; therefore, based on such information, persons in the field of molecular biology would know exactly that the sequence fragment from 672-723 of HUMEF1AB gene is to be amplified in order to construct probe 3.

(See pages 5, 6, 8 and 9 of the application).

As the whole sequences of the exons 7 and 8 of the SMN gene are well known (see page 158 of the Reference #16 in the application – Lefebvre et al. Cell 1995, Vol.80, pp. 155-165), it is not necessary to specify the sequences of the probes 1 and 2 in the

application. The whole sequence of HUMEF1AB gene is also well known in Ann et al., 1988, in Reference #20 in the application; therefore, it is not necessary to specify the sequence of probe 3.

In conclusion, there is no new matter introduced in the disclosure.

May 24, 2004

TO: MS. JEANINE GOLDBERG Fax# (571) 273-0743
U.S. Patent & Trademark Office

FROM: Khue Nguyen, Ph.D.
Tel. (619) 543-3623

RE: Patent Application # 09/938.013

Attached is a draft about the difference between Jong's method and Nguyen's method; this is to facilitate our discussion at our phone conferencing tomorrow morning.

Total fax pages: 3 (including cover page)

Difference between Jong's method and
KV Nguyen's method

Jong's semi quantitative method

Total RNA

↓
RT process using **RANDOM** primer PolydT which results in
cDNA **NON-SPECIFIC**

↓
PCR process using different specific primers which results in different
sequence fragments in different sizes
----- --7-- ------- --------- ---
↓

Performing the sequencing to detect the presence of exon 7 in the above mentioned different PCR fragments. Then, Jong selects the fragments with exon 7 to use for diagnostic purpose. The diagnosis is performed by using the Image Analysis System which shows the intensity of the bands of the PCR, meaning darker or lighter shade of the bands; this technique is called a semi-quantitative analysis method because the information obtained does **not** represent a quantitative value; in other words, Jong's method does not offer precise diagnostic information.

KV Nguyen's quantitative method

Total RNA

↓
RT process using **SPECIFIC** primer (a) which recognizes the **SPECIFIC**
mRNA/SMN; this results in **cDNA/SMN**

---- cDNA/SMN
↓
-PCR process using the **SPECIFIC** primers (c) and (d) to amplify the sequence fragments between exons 5 to 8.

-Construction of the probes:
From the obtained fragments 5 to 8, by PCR:
- using the primers (g) and (h) to amplify a portion of exon 7
- using the primers (i) and (d) to amplify a portion of exon 8

The portion of exon 7 is labeled in two ways – with radioactive label and with biotin label (non radioactive) to get probes 1.

The portion of exon 8 is labeled in two ways – with radioactive label and with biotin label (non radioactive) to get probes 2.

The probes 1 and 2 are used to identify the presence or absence of exons 7 and 8 in normal subjects and SMA patients.

Nguyen's method provides a **quantitative value** for the diagnosis of SMA patients, i.e., it gives specific information in terms of the numbers of exons 7 and 8 in different subjects. For example, normal subjects have a great number of exons 7 and 8; SMA patients have a very small number of exons 7 and 8. Nguyen's method is a quantitative method.

Jong's method is based on the sequencing result in order to identify the PCR fragments containing exon 7 for diagnostic purpose. Different from Jong's method, Nguyen's method is based on the use of SPECIFIC probes to DIRECTLY identify the presence or absence of exons 7 and 8 in different types of subjects for diagnostic purpose.

Different from Jong's semi quantitative method, Nguyen's quantitative method leads to results that offer a quantitative value, which means precise diagnostic information in terms of the **numbers** of exons 7 and 8 versus Jong's method of examining lighter or darker shade of bands of PCR fragments.

Nguyen invented the method of synthesizing the probes specifically for SMA diagnostic purpose.

Nguyen's quantitative method is thus an obvious advancement in the procedure to diagnose SMA.

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May 28, 2004

TO: MS. JEANINE GOLDBERG
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Fax#: (571) 273-0743

FROM: Khue Nguyen, Ph.D.
2828 University Ave., #303
San Diego, CA 92104

Tel. (619) 543-3623; (619) 299-0449)

RE: Patent Application # 09/938,013

Attached is a confirmation of and follow-up on our May 26, 2004 three-way phone conferencing (Jeanine Goldberg, Mai Nguyen and Khue Nguyen).

Total fax pages: 4 (including cover page)

After my clarification for you regarding the difference between Jong's method and Nguyen's method, you have accepted that my method/work is NOVEL. On the criteria of "not routine", we have also agreed that this should not be in relation to all other diseases, and that the consideration here must be specifically related to SMA disease. No one before me has invented the quantitative method specifically designed for SMA diagnostic purpose, i.e., providing quantitative value, precise SMA diagnostic information; no one before me has invented the method of synthesizing the probes specifically for SMA diagnostic purpose. Therefore, my method is clearly innovative; it is "not routine".

You then asked why a quantitative method is needed in SMA diagnosis. I briefly explained that the matter is related to the complex issue of gene duplication and referred you to the information I had previously sent to you. You said you would next read the art again about duplication of genes. For your information, please find attached a summary of background information that lead to why a quantitative method is needed for SMA diagnosis. Along this line, I am sure you know that, in the scientific field, one always strives to find ways to obtain accurate quantitative value; therefore, a quantitative method in itself is an invaluable advancement for research.

It was pointed out that the claiming section needs to be revised in order to be in appropriate technical format. On this point, I would like to thank you for offering suggestions; I look forward to receiving your recommendations based on which I will make the proper revision.

I would like to take this opportunity to thank you and to acknowledge your hard task on this case because it involves an inherently complex genetic disease, the second most fatal autosomal recessive disorder after cystic fibrosis. Because of the difficulty of the topic, it has been necessary for opportunities to explain and clarify, which I think should not affect the evaluation process of my invention.

Characteristics of SMA disease

Spinal Muscular Atrophy (SMA) is characterized by the degeneration of the anterior horn cells of the spinal cord, leading to progressive symmetrical limb and trunk paralysis associated with muscular atrophy. SMA represents the second most common fatal autosomal recessive disorder after cystic fibrosis (1 in 6000 newborn) and is one of the most common genetic causes of death in childhood (Roberts et al., Arch. Dis. Child, 1970, 45, 33-38). Three different forms of SMA: I, II and III, have been recognized on the basis of age of onset and progression of the disease.

Characteristics of SMN gene

The gene most highly associated with SMA is the survival motor neuron (SMN) gene, located in the chromosome 5q13.3. The SMN gene is composed of 8 exons extending over 20 kb and has telomeric (SMNT) and centromeric (SMNC) copies that are highly **homologous**. **The SMNT and its centromeric copy (SMNC) differ in their exons by only two base pairs, one in exon 7 and one in exon 8.** This difference allows the distinction of the SMNT gene from SMNC by single-strain conformation polymorphism (SSCP) analysis (Lefebvre et al., Cell, 1995, 80, 155-165), or by use of restriction enzymes (Van Der Steege et al., Lancet, 1995, 345, 985-986).

Circumstances under which SMA disease occurs

SMA disease occurs when there is deletion of SMNT or mutation of SMNT:

A. Only homozygous absence of SMNT is responsible for SMA, while homozygous absence of SMNC, found in 5% of control population, has no-clinical phenotype. The presence of the SMNC copy hampers detection of absence of the SMNT gene. It was reported that 93% of all types (I, II & III) of SMA patients carry homozygous deletions of exons 7 and 8 or only exon 7 of the SMNT gene. This deletion is due to either the result of SMNT gene deletions or due to the conversion of sequences in the SMNT gene to those in the SMNC gene (Lefebvre et al., Cell, 1995, 80, 155-165; DiDonato et al., Ann. of Neurol., 1997, 41, 230-237).

B. Small mutations in the SMNT also cause the disease -- Several small mutations in the SMNT gene have been reported in patients without a deleted or sequence-converted SMNT gene (Lefebvre et al., Cell, 1995, 80, 155-165; Bussaglia et al., Nature Genet., 1995, 11, 335-337).

SMA diagnosis

The standard polymerase chain reaction (PCR) assay method currently used by most clinical laboratories to confirm the diagnosis of SMA takes advantage of the base differences in exon 7 and exon 8 to distinguish between the SMNT and SMNC genes by means of SSCP analysis (Lefebvre et al., Cell, 1995, 80, 155-165), or by use of restriction enzymes (Van Der Steege et al., Lancet, 1995, 345, 985-986). These

qualitative standard methods for molecular diagnosis of SMA at the DNA level can be used to determine whether an individual lacks both copies of exon 7 (and/or exon 8) of the SMNT gene. However, **the qualitative methods cannot be used to detect non-deletional mutations, such as point mutation, nor to identify SMA carriers.**

Thus, the development of another approach for accurate quantitative molecular diagnosis of SMA is needed. For such a purpose, I developed a **quantitative** method for molecular diagnosis of SMA at the mRNA level. It was reported that the mRNA/SMNT were absent and that the mRNA/SMNC were solely present in the patients lacking the SMNT gene on both mutant chromosomes, while control individuals expressed both mRNA/SMNT and mRNA/SMNC. The majority of mRNA/SMNT are full length, incorporating exon 7. However, major mRNA/SMNC have a high degree of alternative splicing and tend to show little or no exon 7 (Lefebvre et al., Cell, 1995, 80, 155-165). Taking into account the above mentioned problem, I developed the quantitative method based on the measurement of specific mRNA: mRNA/SMNT and mRNA/SMNC by using the specific labeled nucleotide probes 1 and 2 directed at exon 7 and exon 8 respectively. The quantitative method allows the identification of the numbers of transcripts (mRNA) specific to SMN, in order to distinguish between normal subjects, SMA patients and SMA carriers. Normal subjects have the highest number of transcripts containing exons 7 and 8, SMA patients the lowest; SMA carriers's number of transcripts are in between.